THE EFFECTS OF ACTH ON THE ACTIVITY OF SUCCINIC DEHYDROGENASE DURING TAIL PROLIFERATION IN RANA CATESBEIANA LARVAE

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ABSTRACT

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The Effects of ACTH on the Activity of Succinic Dehydrogenase During Tail Proliferation in Rana Catesbeiana Larvae

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This study was undertaken to determine the effects of ACTH on the activity and concentration of succinic dehydrogenase (SDH) and to find out if there was a direct or indirect relationship between enzyme concentration and activity in the proliferating tails of Rana catesbeiana larvae. Tissue homogenates were prepared from the tails (2-6 cm long) 2 hr after the oral administration of ACTH. The homogenate was incubated at 37 C in a test tube which contained a 0.1 per cent solution of triphenyltetrazolium chloride, 0.1 M phosphate buffer (pH 7.4), 0.2 M sodium succinate and distilled water. The optical density was read at 420 mu on the supernatant.

The ACTH produced a significant decrease in SDH activity in tails 5-6 cm long. A significant decrease was observed in SDH concentration in tails 2-3 cm in length. No direct or indirect relationship was found to exist between the enzyme concentration and activity.

It was concluded that the observed decrease in SDH concentration and activity were produced as a result of the stimulation of the adrenal cortex by ACTH to inhibit glucose utilization by the cells.

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CHAPTER I

INTRODUCTION

It has been demonstrated that enzyme activity may be altered by a number of physical and chemical means. Among these are temperature and pH (Jencks, 1963), cortisone (Cerbon and Tamayo, 1959), and ethyl carbamate (Walton, 1966). Changes in the activity of a particular enzyme may be produced in several ways. Some of these are: (1) denaturization of the enzyme, (2) feedback or end-product inhibition, (3) control of the level of the required co-enzyme, and (4) controlling the amount of substrate.

Adrenocorticotrophic hormone (ACTH) stimulates the adrenal cortex to secrete a group of steroid hormones called glucocorticoids. The principal role of glucocorticoids is in carbohydrate metabolism. They promote the deposition of liver glycogen and the inhibition of glucose utilization by the cells. They also promote gluconeogenesis, or the conversion of body proteins to sugar. Succinic dehydrogenase (SDH) is an enzyme located in the mitochondria of the cell. It is an enzyme of the tricarboxylic acid (TCA) cycle and it catalyses the transference of 2 hydrogens to an appropriate hydrogen acceptor, flavin adenine dinucleotide (FAD), during the conversion of succinic acid to fumaric acid.

Recently, a number of studies has been done on the effects of ACTH on various enzymes in a variety of organisms (Palkovic, 1964; Cerbon and Tamayo, 1959). The results of these studies have been

found to be different among animals of the same genus. They have been found to be equally as different in the various organs of the same animal. Because of the many variations in effects produced by ACTH, the present investigation was undertaken to lend some stability, if possible, to those results obtained by earlier investigators. The primary objectives of this study were: (1) to determine the effects of ACTH on the activity of SDH in the proliferating or extending tail of Rana catesbeiana larvae, (2) to determine the effects of ACTH on the concentration of SDH, and (3) to determine if there was a direct or indirect relationship between SDH concentration and SDH activity.

CHAPTER II

REVIEW OF LITERATURE

Only a few studies on the enzymes of the tricarboxylic acid (TCA) cycle in the Amphibia have been reported in the literature. Furthermore, no investigations on the effects of adrenocorticotrophic hormone (ACTH) on TCA cycle enzymes could be found. Therefore, the following review will consist primarily of work done on the activity and concentration of enzymes of glycolysis and the normal activity of those of the TCA cycle.

According to Neilands and Stumpf (1964), Thunberg observed in 1920 that of some 50 compounds tested, citrate, malate, succinate, and fumarate, when added to tissue homogenates, were the most active in reducing methylene blue anaerobically. These initial observations stimulated other workers to search for an explanation. It was not until 1937, according to Neilands and Stumpf, that H. Krebs, aware of these and other data, proposed a hypothesis which could explain all these findings. Since the proposal of the TCA cycle by Krebs, only a small number of studies has been conducted to test the effects of certain chemicals on the enzymes of this cycle, as compared to the large number done on the enzymes of the glycolytic pathway. The studies which have been reported include the use of a variety of organisms, with rats being the choice of most investigators.

There appears to be some controversy as to the exact role of ACTH in carbohydrate metabolism. Many investigators have shown that ACTH

does function in carbohydrate metabolism, but they do not all agree as to how. For example, Rogers (1964) found that the principal effect of ACTH is on glucocorticoid secretions. These secretions function primarily in carbohydrate metabolism. The functions include the deposition of liver glycogen, the promotion of gluconeogenesis, and the inhibition of glucose utilization by the cells. On the other hand, Jeanrenaud and Ho (1967) have demonstrated that ACTH stimulated rather than inhibited the uptake of glucose. They also found that ACTH stimulated the subsequent oxidation of glucose to carbon dioxide. With further regard to the varying opinions of the role of ACTH in carbohydrate metabolism, Koepf (1942), using rat liver slices, found that ACTH increased the synthesis of carbohydrates from pyruvate or d-lactate. Bernhard (1968), in studying the effects of steroids and ACTH on pyruvate and acetate metabolism in sheep adrenal cortex, obtained results similar to those of Jean renaud and Ho. He found that ACTH in concentrations of 1.0 U/ml and 0.1 U/ml resulted in an increase in the incorporation of C^{14} into pyruvate and acetate. (U = 19425 by the international standard for ACTH). Contrary to the findings of all the previously mentioned workers, Overbeek (1950) reported that high doses of ACTH administered to dogs had no effect on carbohydrate metabolism.

Jeanrenaud and Ho (1967) also reported that the total lipid synthesis from glucose to fatty acids was found to be stimulated by ACTH. They suggested that the free fatty acid levels within the adipocyte may represent an intermediate link resulting in the stimulation or inhibition of certain enzymatic activities, both secondarily increasing the uptake of glucose.

In contrast to the studies reported on the role of ACTH in carbohydrate metabolism, those reported on SDH concentration are much more uniform in results. Baker (1963) reported that insect muscles generally show greater enzymatic activity than vertebrate flight muscles and that those indulged in sustained flight possess a greater concentration of SDH. The work of Vallyathan (1964) yielded results which were in accord with the findings of Baker. In studying the effects of exercise on phosphorylase and SDH levels in pigeon breast muscles, he obtained a highly significant increase in concentration and activity of both enzymes. He suggested that during muscular activity the enzyme systems in vivo operate at a considerably high rate in order to keep up with the greater metabolic activity of the muscles. Germino (1965) found greater concentrations of SDH in the earliest stages of development in his study on the developing skeletal muscles of the chick. He interpreted this high concentration to be due to the greater energy requirements of the muscle cells, used mainly in the synthesis of specific proteins.

Attempts to demonstrate hormonal effects on TCA cycle enzymes have thus far been rather limited. This has also been found to be true for enzymes of glycolysis. However, Palkovic (1964) found that there was an increase in glucose-6-phosphate dehydrogenase activity 10 min after various doses of ACTH were administered to rats. He concluded that ACTH stimulation of the adrenal cortex was accompanied by changes in the activity of glucose-6-phosphate metabolizing enzyme. Unlike the findings of Palkovic (1964), Kun and McCurley (1950) had shown that cortisone and ACTH decreased the rate of the enzyme

regulating the formation of phosphopyruvic from 3-phosphoglycerate in rat tissue.

Cerbon and Tamayo (1959) studied the effects of cortisone on the SDH activity of tissues of tubercular (T.B.) animals. Applying tetrazolium colorimetric techniques, these investigators found that tubercular guinea pigs which were not treated with cortisone showed a progressive decrease in SDH activity in the kidney. Those treated with cortisone did not show a decrease in enzymatic activity. Cerbon and Tamayo (1959) suggested that the effects of T.B. on SDH activity was mediated through the adrenal glands, and the possibility exists that the low levels of adrenocortical activity is related to significant metabolic alterations when detected in patients with advanced T. B.

The normal activity of TCA cycle enzymes has been studied by a number of investigators in which a variety of organisms was used. In a histochemical study of SDH activity in the skin of humans, Takada (1963) observed that the activity of SDH had a close relationship with the normal functions of the cells, such as metabolism, growth, maturity and proliferation. In addition, under morbid conditions the activity increased according to cell proliferation and decreased according to regressive degeneration of the skin. In the chick embryo, enzymes of the TCA cycle follow several different patterns. Succinic dehydrogenase activity rises steadily from 7 to 20 days (Davidson, 1957). Glutamic dehydrogenase and malic dehydrogenase rise two to threefold from 7 to 15 days but then decline sharply (Solomon, 1959). Some enzymes do not change in activity at any time. Adenosine deaminase is constant from 7 days to adulthood according to Solomon. Moore

(1964) reported that Potter and his associates in 1945 found that in rat livers, SDH increased rapidly after birth, reaching close to adult levels within 3 days. These increases are interesting in light of the fact that the liver in rats scarcely grows during the first 10 days after birth.

Although studies on the TCA cycle enzymes are virtually absent from the literature in the case of Amphibia, there is little doubt that the cycle does occur in Amphibia. Hunter and Hunter (1957) clearly demonstrated the presence of the TCA cycle in the skin of Rana pipiens. The following enzymes were demonstrated on the basis of oxygen uptake over that of endogenous controls after addition of various cycle intermediates to flasks containing pieces of skin: fumarase (fumarate to malate), malic dehydrogenase (malate to oxalacetate), isocitric dehydrogenase (isocitrate to oxalsuccinate), succinoxidase (succinate to fumarate). In addition, the participation of cytochrome c in oxygen uptake and the presence of glycerophosphate dehydrogenase (glycerophosphate to glyceraldehyde-3-P) were also demonstrated. Weber (1965) did studies on the activity of a number of enzymes, primarily lysosomal, in which he used frog larvae, but his studies were made during tail regression rather than proliferation. However, he showed that proteolytic enzymes in the regressing tail tissue of the frog exhibit high levels of activity. On the other hand, enzymes that are apparently involved in energy metabolism, such as SDH, show a decrease in activity.

CHAPTER III

MATERIALS AND METHODS

Rana catesbeiana larvae were obtained from a pond on Burton Road in Northwest Atlanta, Georgia. The organisms were kept in the laboratory in 12 and 14 gal aquaria and fed boiled lettuce 4 times per week. The water was aerated daily and changed once every 10 days.

Organisms whose tail lengths ranged from 2 to 6 cm during the time when the tail is in the process of extending or proliferating were selected for experimentation. The experimentals were administered 0.2 cc of concentrated ACTH orally with a 2½ cc sterile syringe 2 hr prior to their being sacrificed. Tails 2-6 cm from uninjected organisms were used as controls. (The ACTH was obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio.)

The tadpoles were divided into 4 groups based on the length of their tails:

Group I. Five organisms whose tail length ranged from 2-3 cm were used as experimentals. Four controls were selected whose tail length fell within the same range.

Group II. The 5 experimentals chosen for this group had tails ranging from 3-4 cm. Two controls were selected which had the same range in tail length.

Group III. Again 5 experimentals were selected, along with 3 controls. All tails in this group were from 4-5 cm long.

Group IV. The final group consisted of the same number of

experimentals and controls as were in Group III. However, in this group the tail lengths ranged from 5-6 cm in length.

Succinic dehydrogenase activity and concentration were measured by means of the reduction of triphenyltetrazolium chloride (TTC) to its colorimetrically determinable red formozan, according to the methods described by Kun and Abood (1949). The tails were rapidly excised from pithed tadpoles. They were washed in cold distilled water, blotted dry and weighed on a Mettler analytical balance. The appropriate amount of cold distilled water was poured into a mortar so that once the tissue was homogenized there would be a 10 per cent homogenate. The tails were then placed in the mortar which was in an ice bath. They were minced with a scalpel and ground vigorously with a pestle for 5 min. With a pipette, 1 ml of the homogenate was added to a test tube which contained 0.6 ml of 0.1 per cent solution of TTC, 0.4 ml of distilled water, 0.5 ml of 0.1 M phosphate buffer (pH 7.4), and 0.5 ml of 0.2 M sodium succinate. A blank was prepared which contained all the constituents mentioned above except the homogenate. In its stead, 1 ml of distilled water was added.

The two tubes were incubated in a water bath for 20 min at 37 C. The tubes were removed from the water bath and to each was added a few crystals of sodium hydrosulfite and 7 ml of acetone. The tubes were shaken thoroughly and then centrifuged at 20 x g for 5 min. From each tube was taken 5 ml of the contents which was transferred to colorimeter tubes. The per cent transmittance (per cent T) was read at 420 mμ on a Bausch & Lomb Spectronic 20. The per cent T was converted to optical density (O.D.), since the O.D. is directly proportional to the number

of micrograms of dye reduced. The amount of dye reduced was determined from a standard curve prepared by using a few crystals of sodium hydrosulfite to reduce varying amounts (20 to 400 μg) of TTC (Fig. 1). Enzyme activity was measured according to the number of micrograms of dye reduced in 10 min/mg of tissue. The enzyme concentration was measured according to the number of micrograms of dye reduced in 10 min.

The data obtained were evaluated statistically through the use of the Student "t" test (Little, 1962).

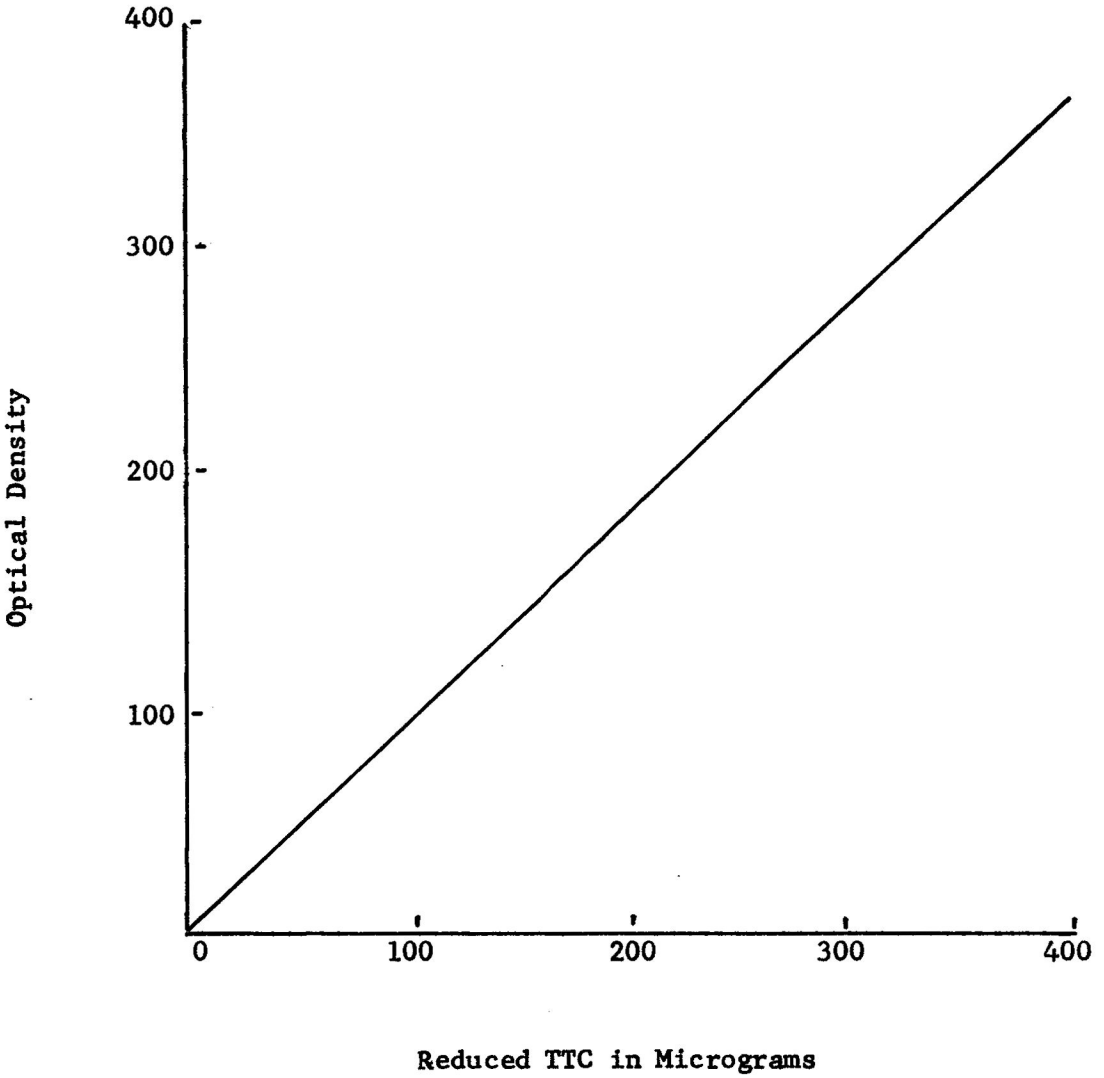

Fig. 1. Standard curve for determining the amount of reduced triphenyltetrazolium chloride.

CHAPTER IV

EXPERIMENTAL RESULTS

Efforts were made to determine the effects of ACTH on the activity and concentration of succinic dehydrogenase (SDH) and the relationship between the enzyme concentration and the enzyme activity.

Effects of ACTH on SDH activity

The data collected showed that ACTH did not have a significant effect on the activity of SDH in the proliferating tails of Rana catesbeiana until the tails had reached a length of from 5-6 cm (Group IV). This is shown graphically in Fig. 2 in which the enzyme activity of the controls and experimentals are quite similar except at the length previously stated. Note that in Group I (2-3 cm) there is a slight increase in activity (experimentals, 0.105; controls, 0.076). The experimentals in Group II (3-4 cm) also showed an increase in activity over the controls (0.251, 0.169). The enzyme activity of the experimentals in Group III (4-5 cm) was less than that found in the controls (0.166, 0.193). The final group, IV, showed a vast difference between the experimentals and the controls (0.062, 0.162). This comparatively large difference was found to be significant at the 0.1 per cent level according to the Student "t" test. Although there are some variations in the average amount of enzyme activity per group between the experimentals and the controls in the first three groups, the Student "t" test showed that these differences were not significant at any level.

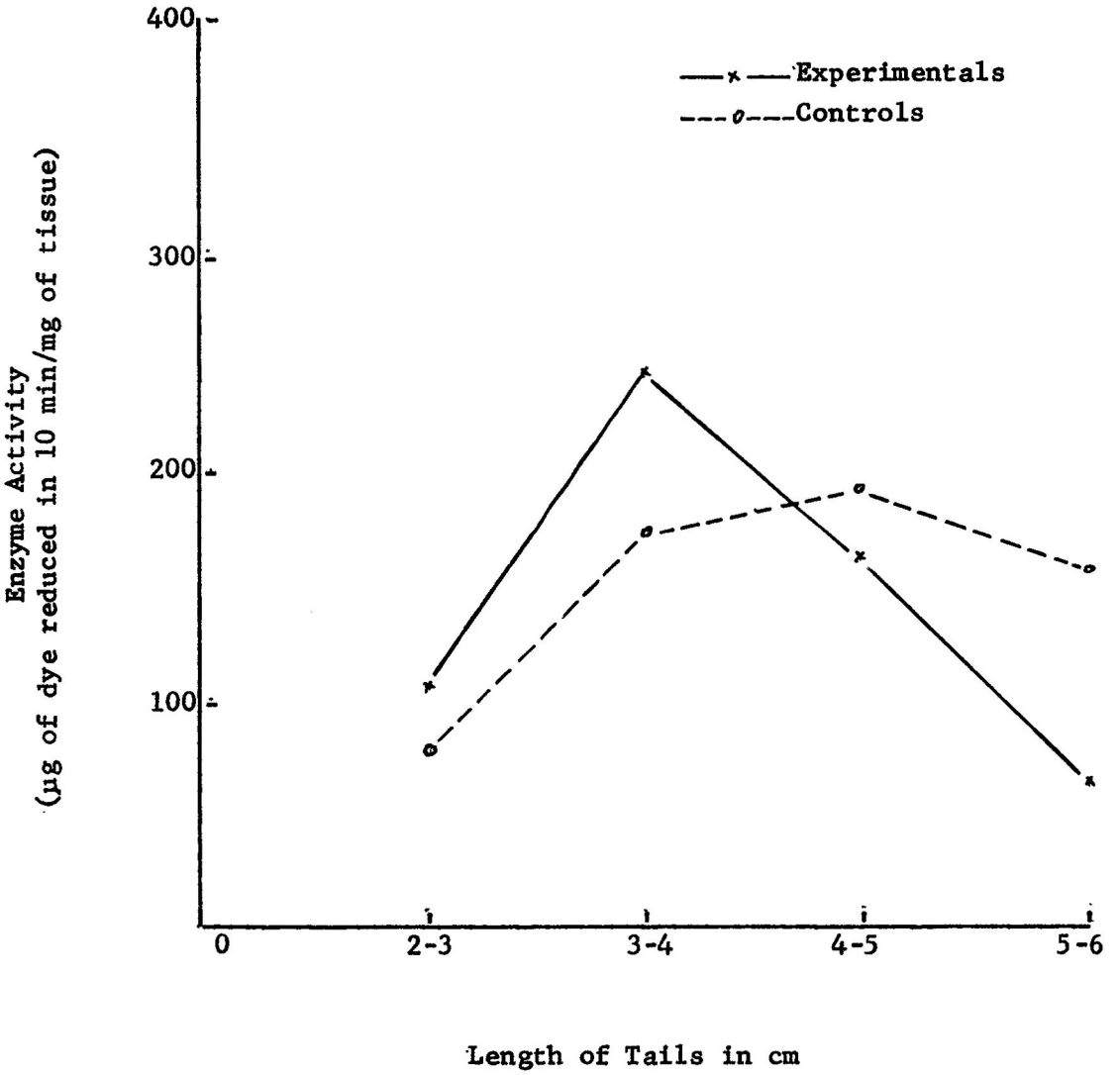

Fig. 2. Graph showing the activity of succinic dehydrogenase in homogenates prepared from varying lengths of proliferating tails of Rana catesbeiana.

Effects of ACTH on SDH concentration

Figure 3 shows a marked difference in the amount of SDH present in the homogenates of tails of the animals which were administered ACTH in Group I as compared with those of the same group which did not receive the ACTH. In the experimentals, the average number of micrograms of TTC reduced in 10 min was 0.116, whereas the TTC reduction in the controls amounted to 0.204 μg in 10 min. In Group II there was only a slight difference in concentration between the experimentals and controls, 0.136 to 0.137. Group III experimentals showed an increase of 0.227 to 0.172 over the controls. The same effect was also true for Group IV, 0.234 to 0.195.

The difference in enzyme concentration in Group I was found to be significant at the 0.1 per cent level, again according to the Student "t" test. Those differences shown in the latter three groups were not found to be significantly different according to the same test.

Relationship of enzyme concentration to activity

During the period of growth when the tails are from 2-3 cm long in the experimentals (Table 1), the enzyme concentration and activity are similar. At 3-4 cm the increase in activity is much greater than the increase in concentration. An increase in concentration occurs when the tails attain a length of 4-5 cm. A decrease in activity occurs at the same length. In the final stages of tail proliferation, when the tails are from 5-6 cm, there is a small increase in SDH concentration and a drastic decrease in activity.

The data obtained on the controls (Table 1) show that the greatest difference in SDH concentration and activity occurred in the earliest

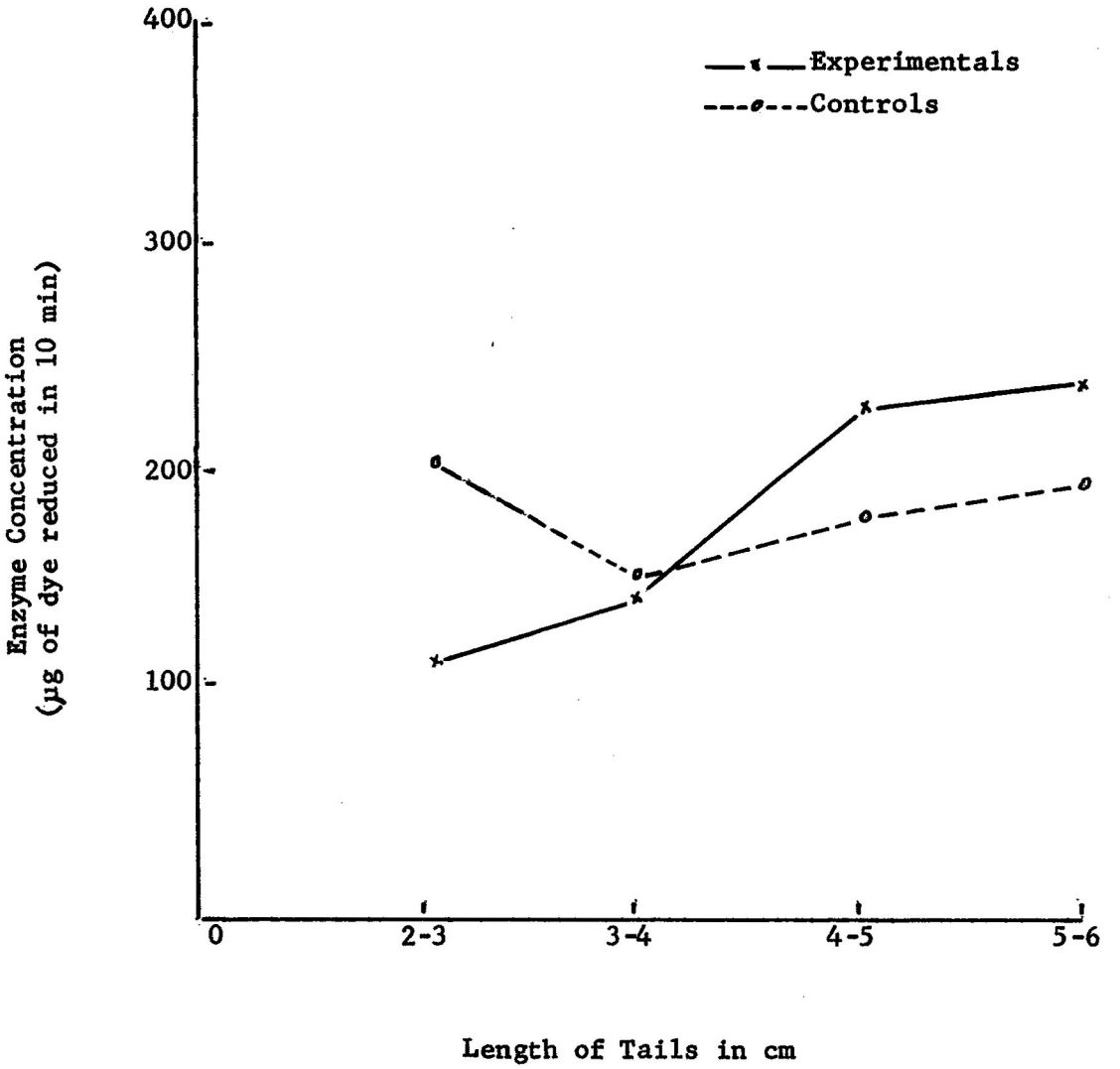

Fig. 3. Graph showing the concentration of succinic dehydrogenase in homogenates prepared from varying lengths of proliferating tails in Rana catesbeiana.

TABLE 1

A COMPARISON OF SDH CONCENTRATION AND ACTIVITY IN THE CONTROLS AND EXPERIMENTALS

Group	Length of tail (cm)	Experimentals		Controls	
		Concentration	Activity	Concentration	Activity
I	2-3	.116	.105	.204	.076*
II	3-4	.136	.251*	.137	.169
III	4-5	.227	.166	.172	.193
IV	5-6	.234	.062*	.195	.162

*These three sets of values for concentration and activity were found to be significantly different. Each value represents the average amount of enzyme activity or concentration per group.

stages of tail proliferation, 2-3 cm. Group I of Table 1 shows the concentration to be 0.204 and the activity to be 0.076. The concentration drops to 0.137 at a tail length of 3-4 cm, but the activity rises to a point slightly greater than the concentration, 0.169. Organisms of Group III (4-5 cm) showed a small increase in concentration and activity to 0.172 and 0.193, respectively. At 5-6 cm the concentration of the controls continued to increase moderately to 0.195, as the activity dropped to 0.162.

The patterns of SDH activity and concentration in the experimentals was distinctly different from those found in the controls. The major differences in activity and concentration occurred at tail lengths of 3-4 and 5-6 cm in the experimentals but in the controls the major differences occurred in tails 2-3 cm long. A statistical analysis of these data showed that the above mentioned differences

were significant at the 0.1 per cent level. Overall, the experimentals showed a moderate but steady increase in SDH concentration as the tails proliferated. On the other hand, the activity of the experimentals rose sharply at 2-3 and 3-4 cm. It fell equally as sharp at 4-5 and 5-6 cm. The concentration in the controls showed a moderate and steady increase only after the tails had reached a length of 3-4 cm. Note in Table 1 that the SDH activity in the controls increased through the first three groups (2-5cm) and then decreased only slightly when the tails were from 5-6 cm long.

CHAPTER V

DISCUSSION AND CONCLUSION

Because of the relatively small number of studies reported in the literature on enzymes of the tricarboxylic acid (TCA) cycle and the absence of studies on these enzymes with ACTH as the treatment, it is felt that more information in both cases is needed. It is hoped that this investigation, in which the effects of ACTH on the activity of succinic dehydrogenase (SDH) were measured, will provide some information pertaining to the TCA cycle enzymes and the effects of hormones on them.

In the present investigation, it was found that ACTH produced a significant decrease in SDH activity in homogenates of tails 5-6 cm long. A slight increase was found in tails 2-3 cm and 3-4 cm, whereas a small decrease was found in tails 4-5 cm. It was shown statistically that the latter three changes in activity were not significant. The significant decrease in enzyme activity reported here is in agreement with the findings of Kun and McCurley (1950) with regard to the changes in enzyme activity produced by ACTH. They showed that cortisone, a glucocorticoid, and ACTH decreased the rate of the enzyme regulating the formation of phosphopyruvic from 3-phosphoglycerate in rat tissue. Although Palkovic (1964) studied an enzyme of a different system from the one reported in this study, he showed that ACTH increased the activity of glucose-6-phosphate dehydrogenase in rats. Baldwin (1967) suggested that inhibition or inactivation of enzymes

was often brought about by chemical agents that attack the reactive groups of the enzyme. Vallyathan (1964) interpreted increased and decreased enzyme activity to be due to the use or disuse of an organ. In agreement with this view is Cherian (1965), who in studying SDH activity in pigeon breast muscles during 1 to 60 days of disuse of the wings, found the SDH activity to be lowered by such immobilization. Baker (1963), also in agreement, demonstrated that insect muscles indulged in sustained flight show a greater increase in enzymatic activity over muscles not indulged in sustained flight.

Turner (1967) found that the administration of ACTH to intact subjects is followed by an increase in the production of glucocorticoids such as cortisol and corticosterone. These and other cortical steroids have been found to discourage the use of carbohydrates, presumably by acting with somatotrophin to inhibit the hexokinase reaction. The findings of Rogers (1964) were identical to those of Turner. The exact mechanism by which ACTH inhibited the activity of SDH could not be found. However, one possibility is that the amino group of SDH may have been attacked. This could result in a change in the orientation of the enzyme molecule. It is the opinion of this investigator that the information presented by Turner (1967) and Rogers (1964) best explains the decrease in SDH activity and concentration reported in this thesis. The suggested mechanism is that ACTH stimulates the adrenal cortex to produce and secrete glucocorticoids. These secretions in turn inhibit the utilization of glucose by the cells. It is felt that with the reduction in the normal amount of glucose used by the cells, the products of glycolysis and the intermediates of the TCA

cycle will also be reduced. This would result in a reduced amount of the substrate, succinic acid. Therefore, the activity of SDH would be lowered.

In connection with the effects of ACTH on SDH concentration, a significant decrease was found in Group I where the tails were from 2-3 cm long. The mechanism for this decrease in SDH concentration is the same as that suggested for the decrease produced in SDH activity.

There was no direct or indirect relationship found between SDH concentration and activity in the experimentals or controls. This suggests that SDH activity is not directly or inversely proportional to SDH concentration and that although the enzyme may be present in relatively high concentrations, it does not have to be proportionately as active.

CHAPTER VI

SUMMARY

1. ACTH did not have a significant affect on the activity of SDH in the proliferating tails of Rana catesbeiana until the tails had reached a length of from 5 to 6 cm. Here the activity was found to decrease.
2. The concentration of SDH was increased in the tails 2 to 3 cm long after the tadpoles had been administered ACTH.
3. No direct or indirect relationship was found to exist between the concentration of SDH and the activity.

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